



Figure S2 Expression of central spindle proteins in CPC RNAi or sub mutants examined by Western blot. Protein samples from mature oocytes were probed on four separate blots with anti-Incenp, anti-Aurora B, anti-Subito, and anti-tubulin as a loading control. Incenp expression is practically undetectable in the *Incenp* RNAi sample, Aurora B expression is practically undetectable in the *ial* RNAi sample, and Subito expression is undetectable in the *sub* mutant. Aurora B expression is also decreased in the *Incenp* RNAi sample and increased in the *Incenp*^{myc} sample, suggesting that Aurora B is stabilized by the presence of Incenp. Approximately 2.5-fold more material was loaded of the *ial* RNAi and *Incenp* RNAi samples relative to the other samples, except in the anti-Subito blot in which all samples were loaded evenly. Method: Protein samples were prepared by collecting stage 14 oocytes by the same method used for immunostaining, but instead of fixation, oocytes were weighed and SDS gel loading buffer was added to obtain a final concentration of 1 mg oocytes/8 μ L total volume. The mixture was boiled for five minutes and 8 μ L was loaded per lane (20 μ L was loaded for *ial* RNAi and *Incenp* RNAi samples) on an SDS-PAGE gel. Primary antibodies used were rat anti-Incenp (1:10,000), rabbit anti-Aurora B (1:5000) (GIET and GLOVER 2001), rat anti-Subito (1:4000) (JANG *et al.* 2005), rabbit anti-KLP10A (1:10,000,000) (ROGERS *et al.* 2004) and rat anti- α tubulin (1:4000, clone YOL 1/34, Millipore, Billerica, MA, USA). Secondary antibodies used were goat anti-rabbit-HRP and goat anti-rat-HRP (both 1:5000, Jackson Immunoresearch), detected using ECL Plus (Amersham, Piscataway, NJ, USA).